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## Combustion Method for Assay of Biological Materials Labeled with Carbon-14 or Tritium, or Double-Labeled

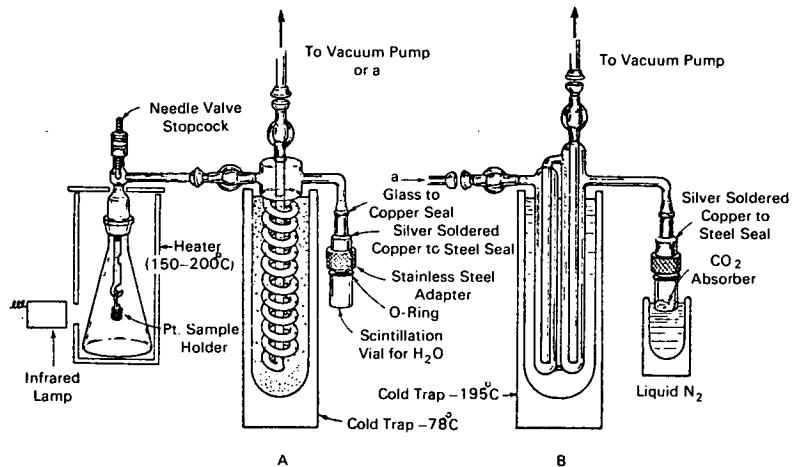


Fig. 1. — Diagram of direct in-vial isotope-collection apparatus for double-labeled samples. (A) Combustion apparatus and collection assembly for  $\text{H}_2\text{O}$ . (B) collection assembly for  $\text{CO}_2$  or  $\text{SO}_2$

### The problem:

Development of an accurate combustion method for assay of biological materials labeled with carbon-14 or tritium, or double-labeled. Measurement and analysis of these low-energy beta emitters, in the presence of various biological materials, can be handled effectively only by liquid-scintillation counting. With biological materials the chief limitations in liquid-scintillation counting are sample dissolution and color quenching, which reduce counting efficiency. If quenching is not excessive, it can be corrected; if excessive, however, the samples must be completely oxidized.

### The solution:

Dry catalytic combustion at high temperatures has proved to be the most accurate method for assay of such labeled materials; it overcomes the problems

of sample solubility and color quenching in liquid-scintillation counting.

The combustion method is a modification of the oxygen-flask technique which provided convenient combustion with the quantitative collection of the resultant  $\text{CO}_2$ ,  $\text{SO}_2$ , and  $\text{H}_2\text{O}$  for subsequent liquid-scintillation measurement. The modified technique combines this method with the increased efficiency and reproducibility of standard vacuum-line techniques; it includes the convenience of direct in-vial collection of the final combustion products, giving quantitative recovery of tritium and carbon-14.

In double-labeled compounds, active combustion products are also quantitatively separated. The main advantage of the technique for counting double-labeled samples is that only a single channel is necessary for counting, and instrument settings and calibration procedures are not critical; only simple

(continued overleaf)

mathematical analysis of the data is necessary, since quenching corrections are eliminated.

#### How it's done:

The apparatus for the combustion method (Fig. 1) consists of a 500-ml combustion flask housed in a heating jacket, a spiral cold trap (*A*) used for collection of water, and a dual trap (*B*) used for collection of carbon dioxide. A standard scintillation vial is attached to each assembly by means of a specially constructed connector and a copper-to-glass seal. In the case of double-labeled samples, such as with carbon-14 and tritium, the cold traps *A* and *B* are connected in series, and the vacuum pump is connected to trap *B*.

Recovery values for tritium from intestine, liver, and blood tissues, with samples weighing up to 100 mg of dry tissue, average 98%; they are reproducible and independent of both the amount and nature of the tissue analyzed. Though the quenching factor increases slightly with increasing weight of tissue, it is constant and reproducible at any set weight of tissue. Thus, where accuracy of a few percent can be tolerated, or where only relative counting rates are required, consideration of quenching can be omitted.

The recovery values for carbon-14 are also about 98%. The efficiency of separation of carbon-14 and tritium is quite satisfactory, with recovery values

similar to those observed when only the single isotope is measured.

#### Notes:

1. For more detail see W. E. Kisieleski and L. G. Huebner, *ANL-7409* (Argonne National Laboratory, March 1967).
2. Medical-research laboratories and food and pharmaceutical companies may be interested.
3. Inquiries may be directed to:

Office of Industrial Cooperation  
Argonne National Laboratory  
9700 South Cass Avenue  
Argonne, Illinois 60439  
Reference: B69-10208

Source: W. E. Kisieleski and L. G. Huebner  
Biological and Medical Research Division  
Argonne National Laboratory  
(ARG-10331)

#### Patent status:

Inquiries concerning rights for commercial use of this innovation may be made to:

Mr. George H. Lee, Chief  
Chicago Patent Group  
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9800 South Cass Avenue  
Argonne, Illinois 60439